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ORIGINAL ARTICLE

Inclusion of fish or fish oil in weight-loss diets for young adults: effects on blood lipids

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Objective: To assess the effects of fish (lean or oily) and fish oil consumption on blood lipid concentration during weight loss.
Design: Randomized, controlled 8-week trial of energy-restricted diet varying in fish and fish oil content. Subjects, 324 men and women, aged 20–40 years, body mass index 27.5–32.5 $kg\,m^{-2}$, from Iceland, Spain and Ireland, were randomized to one of four groups: (1) control (sunflower oil capsules, no seafood), (2) cod diet ($3 \times 150\,g\,week^{-1}$), (3) salmon diet ($3 \times 150\,g\,week^{-1}$), (4) fish oil (DHA/EPA capsules, no seafood). The macronutrient composition of the diets was similar between the groups and the capsule groups were single-blinded.
Measurements: Total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein cholesterol, triacylglycerol (TG) and anthropometrics were measured at baseline and end point.
Results: The difference in logTG lowering between the control group and the cod diet, salmon diet and fish oil from baseline to end point was −0.036 (95% CI −0.079 to 0.006), −0.060 (−0.101 to −0.018) and −0.037 (−0.079 to 0.006), respectively. Reduction in TC was about 0.2 $mmol\,l^{-1}$ greater in the fish groups (cod and salmon) than in the control group, but only of borderline significance when adjusting for weight loss. HDL tended to decrease less in the diet groups consuming a significant amount of n-3 fatty acids (salmon and fish oil).
Conclusion: Weight-loss diet including oily fish resulted in greater TG reduction than did a diet without fish or fish oil. Controlled trials using whole fish as a test meal are encouraged to be able to elucidate the role of different constituents of fish for human health.
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Introduction

It is well known that excessive body weight increases the risk of developing a number of serious diet-related chronic illnesses, including cardiovascular diseases. In this context, countries with high fish intake have been shown to have lower rates of coronary heart disease, compared with other countries.[1–3]

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Studies testing the effects of fish as food in combination with weight loss are scarce.[4,5] Long-chain n-3 poly-unsaturated fatty acids (LC n-3 PUFAs), given as supplements, have in recent trials been shown to have beneficial effects on cardiovascular disease risk factors as an additional therapy to calorie restriction in overweight and obese individuals.[6–8] The triacylglycerol (TG) lowering effects of n-3 fatty acids are well documented,[9] and the beneficial effects of fish intake seen on blood lipids in previous trials have primarily been attributed to the presence of n-3 fatty acids. The role of other seafood constituents, such as fish proteins, has been studied in animals.[10–12] Hypocholesterolemic effects of fish proteins have been reported in animals together with lower hepatic TG concentration.[13] Very few human trials have addressed this issue,[14] and studies on the effects of lean fish intake in comparison with oily fish intake on blood lipids during weight loss are very limited.[15]

Emphasis should be on obesity prevention in children not only to prevent serious consequences of overweight and obesity, but also to study effects of different weight loss diets especially among young people. In this perspective, known risk factors for chronic diseases often associated with overweight and obesity should also be assessed. By means of a randomized controlled trial in three European countries, the aim of this study was to investigate the specific effects of seafood consumption on blood lipids during weight loss induced by an energy-restricted diet in young European overweight individuals. This was done by comparing an energy-restricted control diet, without food of marine origin, with energy-restricted diets including either fish or fish oil, all similar in composition with regard to total fat, protein and carbohydrate content.

Subjects and methods

The study was a randomized 8-week intervention trial of four energy-restricted diets in three European countries: Iceland, Spain and Ireland. The study is part of a large multi-centre study, funded by the European Union: SEAFOODplus—a better life with seafood.

Participants

Subjects were recruited through advertisements. The enrolment of 320 subjects (140 from Iceland, 120 from Spain and 60 from Ireland) was planned. Initially 324 subjects (138 men and 186 women) were included. The Icelandic and Irish subjects were of Caucasian origin and the Spanish participants were Hispanic. The inclusion criteria were body mass index 27.5–32.5 $kg\,m^{-2}$, age 20–40 years and waist circumference of $\geqslant$94 and $\geqslant$80 cm for men and women, respectively. Participants were students at the local universities and employees at major companies and institutions in Reykjavik, Cork and Pamplona. Majority of the subjects were married or in common-law marriage (Table 2), and 60–80% of subjects were studying in universities, had a university degree or higher education. Exclusion criteria were weight change due to weight-loss diet within 3 months before the start of the study, use of supplements containing n-3 fatty acids, calcium or vitamin-D during the last 3 months, drug treatment of diabetes mellitus, hypertension or hyperlipidemia and women's pregnancy or lactation. Recruitment was undertaken in 2004 and 2005. The study was approved by the National Bioethical Committee in Iceland (04-031), the Ethical Committee of the University of Navarra in Spain (24/2004) and the Clinical Research Ethics Committee of the Cork University Hospital in Ireland. The study followed the Helsinki guidelines, and all subjects participating gave their written consent before the start.

Protocol

The subjects made at least three visits to the nutrition unit during the 8-week trial, at baseline, midpoint and end point. Additionally, they were contacted on at least two other occasions by phone and/or email, in weeks two and six. At baseline, midpoint and end-point visits, they met a dietician, anthropometrical measurements were performed and seafood intake was assessed by a validated food frequency questionnaire described elsewhere.[16] Dietary intake was assessed by 2-day weighed food records before baseline (habitual diet) and in week six or later during the two last weeks of the intervention trial. At baseline, information on physical activity patterns during the last year, smoking habits, alcohol consumption and use of contraceptive therapy was collected using a questionnaire. Subject's physical activity level was assessed at baseline and end point and subjects were instructed not to change their physical activity levels. Subjects were instructed to keep their alcohol consumption to a minimum. The dietary records were analysed using the food-nutrient database in each country. Compliance to the diets was assessed by the 2-day weighed food records, by the validated food frequency questionnaire and by analysing n-3 and n-6 fatty acids in erythrocyte phospholipids in fasting blood samples from the subjects.

Randomization and control group (placebo)

After baseline experimentation, each volunteer was randomly assigned to one of the four experimental diets (1: control, 2: cod (lean white fish), 3: salmon (oily fish), 4: fish oil). The randomization list was computer generated (SPSS programme) by the Icelander partner. In each country, the research dietician/nutritionist assigned the randomization number to each subject enrolled into the trial. People following the control and fish oil diets were single-blind supplemented every day with six sunflower oil capsules as placebo and six fish oil capsules, respectively. Participants were provided with cod and salmon during the trial (Table 1). The proximate chemical composition of the fish and capsules (Table 1) used in the study was carried out according to Association of Analytical Communities (AOAC) methods,[17] and fatty acid determination followed the conditions described previously by Bandarra *et al.*[18] Absolute values were calculated using the correction factors proposed by Weihrauch *et al.*[19]

Intervention diets

Basal metabolic rate was estimated by applying Harris–Benedict equations,[20] and a correction factor due to the overweight status of the subjects.[21,22] Ideal body weight was calculated from the following formula: ideal body weight $= 50 + (0.75 \times (\text{height} - 150)$. The weight that was used in the HB equation was then calculated: ((actual weight$-$ideal body weight) $\times$ 0.25) + ideal body weight. To estimate total energy expenditure, the physical activity level was set according to low physical activity level as reported by

Table 1 Description of the intervention diets and the n-3, EPA, DHA and n-6 intake attributable to the fish and capsules used in the different diets (mg day^{-1})[a]

Diet groups	*Amount consumed per day*	*n-3 fatty acids (mg day^{-1})[a]*	*EPA (mg day^{-1})[a]*	*DHA (mg day^{-1})[a]*	*n-6 fatty acids (mg day^{-1})[a]*	*Manufacturer*
Control	Six placebo (sunflower oil) capsules per day	6 ± 0.2	ND	ND	336 ± 5	Loders Croklaan (Lipid Nutrition), Wormerveer, The Netherlands Encapsulated by Banner Pharmacaps, Tilburg, The Netherlands)
Cod	150 g cod three times per week	272 ± 3	54 ± 7	207 ± 12	17 ± 1	Samherji, Iceland
Salmon	150 g salmon three times per week	3004 ± 129	774 ± 29	1370 ± 88	665 ± 20	Marine Harvest, Nutreco, Norway
Fish oil	Six fish oil capsules per day	1418 ± 34	633 ± 9	430 ± 9	124 ± 3	Loders Croklaan (Lipid Nutrition), Wormerveer, The Netherlands. Encapsulated by Banner Pharmacaps, Tilburg, The Netherlands

Abbreviation: ND, not detected. [a]Calculated from fatty acid analysis of samples of fish and capsules ($n = 8$ for each diet). Each subject was instructed to follow a diet, energy-restricted by 30% from estimated energy expenditure. All four diets were designed to provide 30% of total energy from fat, 50% from carbohydrates, 20% of total energy from proteins and dietary fibre 20–25 g day^{-1}.

subjects.[23] Subjects were instructed to follow a diet, energy-restricted by 30% from the estimated energy expenditure (approximately 600 kcal day^{-1}), for 8 consecutive weeks. All four diets were designed to provide 30% of total energy from fat, 50% from carbohydrates, 20% of total energy from proteins and dietary fibre (20–25 g day^{-1}). Each subject got a detailed diet plan, menus and an exchange system to follow for 8 weeks along with detailed instructions from the dieticians/nutritionists to minimize difference between diets in sources of fat (subjects were instructed to use only olive oil and no other oil during the trial), fruit and vegetable consumption and meal frequency. Subjects in the fish groups (cod and salmon) were encouraged to bake the fish rather broiling or frying and were provided with recipes appropriate to their estimated energy level.

Serum lipids
Blood sampling (after overnight fasting) was conducted at clinical settings in each centre. Chemical analysis was performed centrally. Total cholesterol (TC) and TG were analysed using an enzymatic colorimetric assay from Roche automated clinical chemistry analyser 'Hitachi 911', high-density lipoprotein cholesterol (HDL-C) was determined directly in serum, using poly-ethylene glycol-modified enzymes and dextrane sulphate. Levels of low-density lipoprotein cholesterol (LDL-C) were calculated using the Friedwald formula.

Anthropometrical measurements
Anthropometrical measurements were performed on three occasions during the 8-week period: at baseline, midpoint and endpoint. Weight was measured in light underwear on a calibrated scale (SECA 708, Hamburg, Germany) in all centres. Subject's height was measured with a calibrated stadiometer, and waist circumference was measured, using an inelastic measuring tape, following standard methods.[24] All measurements were done using standard measurement procedures as outlined in a research protocol used by all countries participating in the study.

Statistical analysis
The individuals included in the study were young and healthy individuals, except for their being overweight or obese, so a normal model was a natural candidate. With 65 subjects per group (assuming 20% drop-out rate), the study was powered to detect 0.3 s.d. difference in outcome variables at 80% power. In the initial descriptive approach, some univariate models were estimated. Some two-way interactions were tested but they were not significant. The dependent variables were TC, HDL-C, LDL-C and logTG. Two linear models were applied:

$$\Delta = \mathrm{y\ at\ baseline} + \mathrm{gender} + \mathrm{country} + \mathrm{dietary\ groups} \quad (1)$$

$$\Delta = \mathrm{y\ at\ baseline} + \mathrm{gender} + \mathrm{country} + \mathrm{dietary\ groups} + \Delta\,\mathrm{weight} \quad (2)$$

The y variables were TC, HDL-C, LDL-C and logTG. Serum TG concentration was transformed before analysis to reduce skewness in the distribution. Traditional residual diagnosis was performed. The drop-out process was modelled with a binary response model. The impact of the covariates on the probability of dropout was evaluated by a logistic regression.

Results

Baseline characteristics and dropouts
Of the 324 randomized individuals entering the trial, measurements of blood concentration at end point were available for 262 individuals (80%). The flow of participants in the study is shown in Figure 1. The two commonest reasons for dropout were that the subject was unable to follow the prescribed diet and/or lack of time to maintain the

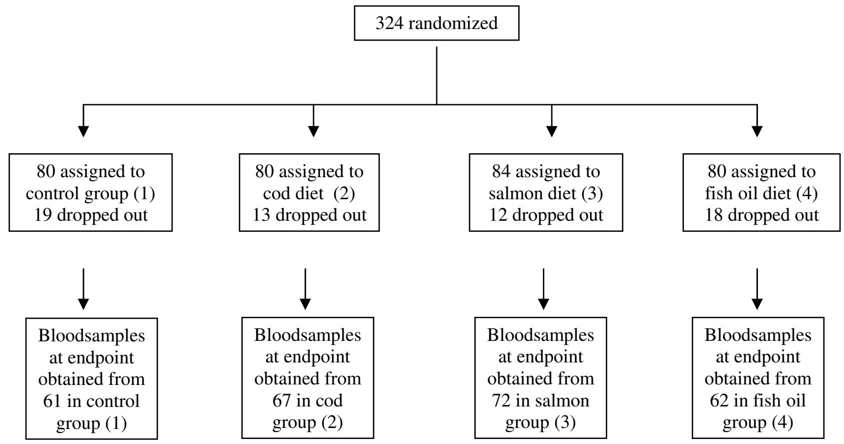

Figure 1 Flow of participants in the study.

Table 2 Baseline characteristics

Women (%)	*Control diet*			*Cod diet*			*Salmon diet*			*Fish oil diet*		
	N	*Mean* 60	*s.d.*	N	*Mean* 56	*s.d.*	N	*Mean* 50	*s.d.*	N	*Mean* 63	*s.d.*
Current smokers (%)		20			23			28			20	
Married or in common-law marriage (%)		86			71			72			78	
Age (years)	80	32.1	5.3	80	31.3	5.7	84	31.3	5.3	80	31.0	5.3
Weight at baseline (kg)	80	87.7	10.1	80	89.5	9.4	84	90.4	11.4	80	85.0	9.4
Height at baseline (m)	80	1.71	0.09	80	1.72	0.08	84	1.72	0.09	80	1.69	0.09
BMI (kg m^{-2})	80	30.0	1.5	80	30.2	1.4	84	30.4	1.4	80	29.9	1.5
Waist circumference (cm)	80	95.2	7.4	80	96.8	6.7	84	96.9	7.9	80	94.0	6.7
Triacylglycerol (mmol l^{-1})	76	1.07	0.59	79	1.31	0.73	80	1.18	0.52	79	1.15	0.73
Cholesterol (mmol l^{-1})	76	5.07	0.87	79	5.26	1.04	80	5.07	0.88	79	4.95	0.95
HDL-cholesterol (mmol l^{-1})	76	1.39	0.36	79	1.32	0.30	80	1.34	0.44	79	1.38	0.32
LDL cholesterol (mmol l^{-1})	76	3.19	0.74	79	3.37	0.96	80	3.19	0.80	79	3.05	0.87

schedule of clinical visits. Dropouts were equally distributed between intervention diet groups, and variables predicting weight loss or changes in any of the blood parameters measured were not different between dropouts and those who completed the trial. The results of the logistic regression suggest no systematic explanation of dropping out. The baseline characteristics of the subjects are shown in Table 2. The average energy intake of subjects before randomization was 2322 kcal day^{-1} where protein, fat and carbohydrates contributed to 17%, 36% and 42% of the total energy intake, respectively. Frequency of fish consumption at baseline was five (median) times per month as main meal and five times per month as bread spread or side dish. No differences were seen between the diet groups in terms of energy intake, distribution of energy-giving nutrients or frequency of fish consumption at baseline.

Weight loss and unadjusted decrease in blood lipid concentration

The average weight loss was 6.5 kg (s.d. = 3.3 kg) for men and 4.2 kg (s.d. = 2.4 kg) for women. This weight loss was in agreement with what could be expected from the prescribed energy intake during the trial, showing good compliance to the dietary advice. The control group lost on average about 1 kg less weight during the 8-week period than did the groups receiving fish or fish oil (cod diet, salmon diet and fish oil diet). The results on weight loss have been reported in detail elsewhere.[25] The 2-day food record, food frequency questionnaire and the analysis of fatty acid profile in erythrocytes confirmed good compliance to the diets with

Table 3 Mean change (unadjusted) in weight and blood lipids from baseline to end point in the 8-week trial

	Control diet		*Cod diet*		*Salmon diet*		*Fish oil diet*	
	Mean	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*
Weight (kg)	−4.37	2.80	−5.35	2.74	−5.48	3.32	−5.38	3.23
Total cholesterol (mmol l^{-1})	−0.25	0.71	−0.54	0.75	−0.48	0.62	−0.32	0.63
LDL (mmol l^{-1})	−0.19	0.56	−0.34	0.66	−0.33	0.56	−0.15	0.57
HDL (mmol l^{-1})	−0.04	0.23	−0.09	0.17	−0.03	0.19	−0.08	0.19
Triglycerides (mmol l^{-1})	−0.04	0.45	−0.28	0.51	−0.26	0.44	−0.20	0.61

regard to energy intake, fish consumption and distribution of energy-giving nutrients. Physical activity level of subjects did not change during the trial.[25] The average weight loss and unadjusted decrease in blood lipid concentration during the 8-week trial can be seen in Table 3.

Linear models

In all models estimated, the impact of serum lipids reduction was greater in individuals with high serum concentrations at baseline. Baseline concentration was therefore accounted for in all linear models. Changes in body weight during the trial also strongly predicted reduction in blood lipids. The control group lost on average about 1 kg less weight than the other groups. Owing to difference in weight loss between dietary groups, we chose to show the estimates both including and excluding weight loss from the models. Table 4 shows the change in serum lipid concentrations in each dietary group when compared with the control group.

Triacylglycerol

The most significant diet group effect was seen for serum TG. When compared with the control group, the groups receiving the cod diet, salmon diet or fish oil experienced greater reduction in TG concentration (expressed as logTG in Table 4). The cod diet (lean fish) lowered TG to a similar degree as fish oil diet. Interestingly, when including weight loss in the models, it attenuated the estimates for diet group effects only to a minor degree but resulted in a non-significant difference from control for the group receiving cod and fish oil. No significant country effects were observed. A small gender effect was seen, where the TG-lowering effect of the weight-loss diets seemed slightly greater in women than men (adjusted for baseline values and weight loss; data not shown).

Total cholesterol

As seen in Table 4, the reduction in TC was greater in the groups receiving fish (cod or salmon), but not in the fish oil group, compared with the control group, although this difference did not reach statistical significance in model 2 (Table 4) when adjusting for weight loss during the trial. During the 8-week intervention, 1 $mmol\,l^{-1}$ higher baseline value for TC resulted in 0.28 $mmol\,l^{-1}$ (95% CI 0.21–0.36) greater TC reduction. No significant country- or gender-specific effects were observed.

LDL and HDL cholesterol

As seen in Table 4, the cholesterol-lowering effect of the cod diet is partly because of lowering of HDL-C rather than LDL-C, in comparison with the other dietary groups. However, the estimates for LDL tended to be greater in the groups receiving cod or salmon, suggesting that part of the

Table 4 Difference in blood lipid concentration between the control group (receiving no fish or fish oil, only placebo capsules) and the other three dietary groups (cod diet, salmon diet and fish oil diet) assessed by general linear models[a,b]

Model 1[a]	*Difference from control group*	*95% CI*			*Model 2*[b]	*Difference from control group*	*95% CI*		
		Lower	*Upper*	P			*Lower*	*Upper*	P
Total cholesterol ($mmol\,l^{-1}$)					*Total cholesterol*				
Cod diet	−0.243	−0.448	−0.038	0.021	Cod diet	−0.164	−0.352	0.024	0.087
Salmon diet	−0.235	−0.438	−0.032	0.024	Salmon diet	−0.150	−0.337	0.037	0.117
Fish oil diet	−0.106	−0.313	0.101	0.317	Fish oil diet	0.024	−0.166	0.214	0.803
LDL cholesterol ($mmol\,l^{-1}$)					*LDL cholesterol*				
Cod diet	−0.110	−0.289	0.070	0.231	Cod diet	−0.065	−0.238	0.107	0.458
Salmon diet	−0.134	−0.312	0.044	0.141	Salmon diet	−0.079	−0.251	0.092	0.365
Fish oil diet	0.005	−0.177	0.186	0.961	Fish oil diet	0.057	−0.117	0.232	0.521
HDL cholesterol ($mmol\,l^{-1}$)					*HDL cholesterol*				
Cod diet	−0.072	−0.132	−0.012	0.019	Cod diet	−0.061	−0.119	−0.002	0.044
Salmon diet	−0.011	−0.071	0.048	0.711	Salmon diet	0.001	−0.057	0.059	0.970
Fish oil diet	−0.047	−0.108	0.013	0.124	Fish oil diet	−0.037	−0.096	0.022	0.222
HDL/CH (ratio)					*HDL/CH*				
Cod diet	−0.003	−0.016	0.011	0.692	Cod diet	0.005	−0.008	0.018	0.476
Salmon diet	0.011	−0.002	0.024	0.094	Salmon diet	0.009	−0.004	0.022	0.183
Fish oil diet	−0.005	−0.019	0.008	0.458	Fish oil diet	0.007	−0.006	0.021	0.273
logTG ($mmol\,l^{-1}$)					*logTG*				
Cod diet	−0.046	−0.090	−0.003	0.038	Cod diet	−0.036	−0.079	0.006	0.092
Salmon diet	−0.071	−0.114	−0.028	0.001	Salmon diet	−0.060	−0.101	−0.018	0.005
Fish oil diet	−0.047	−0.091	−0.003	0.035	Fish oil diet	−0.037	−0.079	0.006	0.090

[a]Model 1. Variables included are blood lipid concentration at baseline, gender and country. [b]Model 2. Variables included are blood lipid concentration at baseline, gender, country and weight loss during the 8-week trial.

reduction in TC seen in these two groups could be attributable to reduction in LDL-C. HDL-C concentration tended to decrease less in the diet groups consuming a significant amount of LC n-3 PUFAs. No difference was observed between groups in the ratio between HDL-C and TC. A country-specific effect was observed for LDL-C in the model: participants in Spain tended to lower their LDL-C cholesterol $0.16\,\text{mmol}\,\text{l}^{-1}$ (95% CI 0.02–0.30) more than participants in Iceland, adjusted for total weight loss. No gender-specific effect was observed, whereas for HDL-C, no significant country- or gender-specific effect was seen.

Other possible confounding factors

In the linear models presented in Table 4, adjustments were made for baseline concentration of each blood lipid, country, gender and weight loss. Adjustments for other possible confounding factors such as frequency of habitual fish intake or fatty acid profile (% DHA) in erythrocytes at baseline (as an indicator of habitual fish intake), smoking habits, alcohol consumption or contraceptive therapy did not change the findings presented in Table 4, as these factors were equally distributed between the four groups.

Discussion

Inclusion of oily fish in an energy-restricted weight-loss diet among young overweight or obese individuals resulted in greater reduction in TG concentration when compared with a group receiving diet without food of marine origin. Lowering of TC was observed in the fish groups, both cod and salmon, although not statistically significant after adjusting for weight loss during the trial. Reduction of TC observed in the group receiving cod diet (lean fish) was partly because of reduction in HDL-C, receiving relatively low amounts of LC n-3 PUFAs.

Overweight and obesity are increasing around the world and it is therefore very important to study whether different food items in calorie-restricted diets could have different effects on risk factors for chronic diseases during weight loss. This study was designed to assess the effects of inclusion or exclusion of food of marine origin to weight-loss diet. During the past 20 years, several studies have implicated the beneficial health effects of fish consumption.[1–3] These findings are additionally supported by two recent meta-analyses suggesting that fish intake may be an important component for the prevention of coronary heart disease.[26,27] There are many properties in fish that may protect against coronary heart disease,[28] LC n-3 PUFAs being the most frequently involved. LC n-3 PUFAs have been shown to protect against arrhythmia, reduce platelet aggregation and thereby have an anti-thrombotic effect, reduce inflammatory mediators and also to lower triacylglycerol in the blood.[29–33] However, the benefits of LC n-3 PUFAs on cardiovascular health are still a matter of debate.[34]

The TG-lowering effects of LC n-3 PUFAs are well documented.[9,35,36] The hypotriacylglycerolemic effects of LC n-3 PUFAs were apparent in this study, where the greatest reduction in TG concentration was seen in the group receiving the largest amount of LC n-3 PUFAs. The salmon was estimated to provide around 2 g DHA and EPA per day, compared with $1\,\text{g}\,\text{day}^{-1}$ in the amount of fish oil provided, and 150 g of cod eaten three times per week provided about $0.26\,\text{g}\,\text{day}^{-1}$ of DHA and EPA (see Table 1). The fall in TG in the salmon group and the fish oil group is therefore in agreement with the extensive literature on the TG-lowering effects of LC n-3 PUFAs and results from recent trials, showing beneficial effects of LC n-3 PUFA supplementation on cardiovascular risk factors during weight loss.[6–8] However, TG-lowering effect of the lean cod diet shown in this study has not been seen previously in a human study. The different components of fish contributing to cardiovascular health have been assessed in the rat, where cod protein has been shown to lower hepatic TG concentration and TG secretion rate when compared with casein.[13] In a recent human trial by Moore *et al.*,[15] subjects received two portions of oily fish (on average $0.65\,\text{g}\,\text{day}^{-1}$ DHA and EPA) or white fish (on average $0.1\,\text{g}\,\text{day}^{-1}$ DHA and EPA), in combination with either high or low linoleic acid: α-linolenic acid ratio. Dietary intervention by white fish (lean fish) resulted in higher TG levels than the oily fish. It might therefore be suggested that the TG-lowering effect of the cod diet in this trial might be due to the LC n-3 fatty acid content of the diet ($0.26\,\text{g}\,\text{day}^{-1}$ DHA and EPA, by consumption of three 150 g cod meals per week) rather than the fish proteins. However, it cannot be ruled out that the interaction of n-3 fatty acids and proteins (or other nutrients found in whole fish) might be of importance, as the results of this trial suggest that consuming lean fish (cod) three times per week could lower TG concentration to a similar degree as $1.0\,\text{g}\,\text{day}^{-1}$ of DHA and EPA taken as supplements. Controlled trials using whole fish as a test meal are encouraged to be able to elucidate the role of different constituents of fish for human health.

A recent systematic review on the effects of LC n-3 PUFAs on serum markers of cardiovascular disease risk show that the net effect on TC, LDL-C and HDL-C levels are small.[35] In our study, there was no indication on the cholesterol-lowering effect of fish oil. However, in the groups receiving fish (cod and salmon), TC tended to lower more than the groups not receiving fish, although not statistically significant after allowing for total weight loss. The sample size was powered to detect a 0.3 s.d. difference in cholesterol change between diet groups; thus the study was underpowered to detect the 0.16 and 0.15 difference in TC between the fish groups and the control group in model two (see Table 4). In previous studies in rats, fish proteins have been found to decrease plasma TC concentration in comparison with casein.[10–12] Hypocholesterolemic effect of fish protein in rats[13] has been attributed to a decrease in liver cholesterol output into the circulation due to a stimulation of cholesterol to bile acid conversion and to increased excretion of cholesterol and its metabolites into faeces[37] or reduced activity of Acyl-CoA: cholesterol acyltransferace.[38] These observations suggest that fish proteins might have cardioprotective effect and be involved in the regulation of plasma cholesterol. Human studies are needed.[39]

In this study, the cholesterol-lowering effect in the group receiving the cod diet was at least partly because of a decrease in HDL-C. In a recent systematic review of the literature, it was suggested that fish oil (LC n-3 PUFAs) may be most effective at raising (or stabilizing) HDL-C levels in people whose HDL-C levels would otherwise decrease with time.[35] The current trial showed that HDL-C tended to decrease less in the diet groups consuming a significant amount of fish oil. The fat content of the diets used in this study was around 30% of total energy intake. Fall in HDL-C is usual with a low-fat diet, around or less than 30% of energy.[40,41] It seems, therefore, that LC n-3 PUFAs in combination with high-quality fish proteins could be an additional means to decrease cardiovascular risk during weight loss using diets with relatively low fat content.

A constant limitation to nutrition interventions is the difficulty to manage every single factor that could confound the results such as variation in the choice and frequency of fruits and vegetables, animal and vegetable sources of other proteins and fat, amount and type of alcohol consumption or physical activity. An attempt was made to control dietary intake by providing menus and detailed exchange system in this study. Compliance was verified by information on dietary intake, weight loss and blood samples as far as possible. Other factors such as socioeconomic status, educational level and occupation can also be predictive of the response to a weight-loss programme. Our subjects were highly motivated, well-educated individuals. Most of them had in common being married or in common-law marriage, and subjects claimed that they had good support from their spouse.

In conclusion, oily fish incorporated to a weight-loss diet among young overweight or obese individuals resulted in greater reduction in TG concentration when compared with a group receiving diet without food of marine origin. TG-lowering effects of cod, in combination with relatively low amount of LC n-3 PUFAs, shown in this study might be of importance in understanding the health-related effects of fish. Lowering of TC was also apparent in the groups receiving whole fish. Interaction between LC n-3 PUFAs from fish, fish proteins or other nutrients found in fish might be of relevance. Controlled trials using whole fish as a test meal are encouraged to be able to elucidate the role of different constituents of fish for human health.

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